JC03 Rec'd PCT/PTO 20 JUN 2001

(FORM PTO-1390 (Modified) (REV 11-2000)) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
PG3635USW	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
09/868666	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/EP99/01057	21 December 1999
PRIORITY DATE CLAIMED	
23 December 1998	

TITLE OF INVENTION
PRODUCTION OF PROTEINS

APPLICANT(S) FOR DO/EO/US
Islam et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Copy of PCT Publication cover page
Copy of PCT Request

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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/868666	PCT/EP99/01057	PG3635USW

24. The following fees are submitted:.	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED		NUMBER EXTRA		RATE	
Total claims	26	- 20 =	6	x	$18.00	**$108.00**
Independent claims	2	- 3 =	0	x	$80.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.				☐		**$0.00**
TOTAL OF ABOVE CALCULATIONS					=	**$968.00**
☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.						**$0.00**
SUBTOTAL					=	**$968.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).					+	**$0.00**
TOTAL NATIONAL FEE					=	**$968.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐						**$0.00**
TOTAL FEES ENCLOSED					=	**$968.00**
						Amount to be refunded $
						charged $

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **07-1392** in the amount of **$968.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **968.00** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23347
PATENT TRADEMARK OFFICE

SIGNATURE

Virginia C. Bennett
NAME

37,092
REGISTRATION NUMBER

June 20, 2001
DATE

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09/868666
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of: ISLAM et al.
International Application No.: PCT/EP99/10157
International Filing Date: 21 December 1999
Title: PRODUCTION OF PROTEINS

Honorable Commissioner of Patents
Washington, D.C. 20231

FIRST PRELIMINARY AMENDMENT

Dear Sir:

The above-identified application is being transmitted herewith for entry in the US National Phase under Chapter II of the PCT for the purpose of adding the priority information. Please amend the application as follows:

In the Abstract:

The Abstract has been placed on a separate sheet of paper according to US practice, as required under 37 CFR 1.72(b).

In the Specification:

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. §371 as a United States National Phase Application of International Application No. PCT/EP99/10157 filed 21 December 1999, which claims priority from Great Britain Application No. 9828624.8 filed 23 December 1998.--

In the claims:

Please cancel claims 1-12 without prejudice or disclaimer thereto.

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Please add the following claims:

13. A process for the production of a protein by a cell in culture, comprising:
(a) providing a eukaryotic cell that constitutively produces a protein, and
(b) culturing said eukaryotic cell on a medium comprising an alkanoic acid component selected from the group consisting of:
(i) alkanoic acids,
(ii) salts of alkanoic acids, and
(iii) a combination of alkanoic acids and salts of alkanoic acids,
said alkanoic acid component maintained in the medium at a concentration of less than about 0.1mM.

14. A process according to claim 13 comprising first culturing said eukaryotic cell on a cell culture medium that does not contain an alkanoic acid component, prior to culturing on a medium containing an alkanoic acid component.

15. A process according to claim 13, further comprising separating said protein from the culture medium.

16. A process according to claim 13 where said eukaryotic cell is cultured on said medium comprising an alkanoic acid component for at least ten days.

17. A process according to claim 13, further comprising subculturing the cell culture resulting from step (b), said subculture on medium comprising an alkanoic acid component selected from the group consisting of:
(i) alkanoic acids,
(ii) salts of alkanoic acids, and
(iii) a combination of alkanoic acids and salts of alkanoic acids,
said alkanoic acid component maintained in the subculture medium at a concentration of less than about 0.1mM.

18. A process according to claim 13 wherein said alkanoic acid component is a straight chain C_{2-10}.

19. A process according to claim 13 wherein said alkanoic acid component is a straight chain C_{3-6}.

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20. A process according to claim 13 wherein the alkanoic acid component is selected from the group consisting of butyric acid and metal salts of butyric acid.

21. A process according to claim 13 wherein the alkanoic acid component is sodium butyrate.

22. A process according to claim 13 wherein the concentration of said alkanoic acid component is less than about 0.1mM but greater than about 0.025mM.

23. A process according to claim 13, wherein said concentration of said alkanoic acid component is less than about 0.1mM but greater than about 0.05mM.

24. A process according to claim 13, wherein said concentration of said alkanoic acid component is about 0.075 mM.

25. A process according to claim 13 wherein said eukaryotic cells are selected from non-immunoglobulin secreting mouse myeloma B cells (NSO) and Chinese Hamster Ovary (CHO) cells.

26. A process according to claim 13 wherein said protein is selected from the group consisting of hormones, enzymes, enzyme inhibitors, lymphokines, and immunoglobulins.

27. A process according to claim 13 wherein said protein is an immunoglobulin.

28. A process for the production of a protein by a cell in culture, comprising:

(a) providing a eukaryotic cell that constitutively produces a protein, and

(b) culturing said eukaryotic cell on a medium comprising an alkanoic acid component selected from the group consisting of:

(i) butyric acid,

(ii) metal salts of butyric acid, and

(iii) a combination of butyric acid and a metal salt of butyric acid,

said alkanoic acid component maintained in the medium at a concentration of less than about 0.1mM.

29. A process according to claim 28 comprising first culturing said eukaryotic cell on a cell culture medium that does not contain an alkanoic acid component, prior to culturing on a medium containing an alkanoic acid component.

30. A process according to claim 28, further comprising separating said protein from the culture medium.

31. A process according to claim 28 where said eukaryotic cell is cultured on said medium comprising an alkanoic acid component for at least ten days.

32. A process according to claim 28 wherein the alkanoic acid component is sodium butyrate.

33. A process according to claim 28 wherein the concentration of said alkanoic acid component is less than about 0.1mM but greater than about 0.025mM.

34. A process according to claim 28, wherein said concentration of said alkanoic acid component is less than about 0.1mM but greater than about 0.05mM.

35. A process according to claim 28, wherein said concentration of said alkanoic acid component is about 0.075 mM.

36. A process according to claim 28 wherein said eukaryotic cells are selected from non-immunoglobulin secreting mouse myeloma B cells (NSO) and Chinese Hamster Ovary (CHO) cells.

37. A process according to claim 28 wherein said protein is selected from the group consisting of hormones, enzymes, enzyme inhibitors, lymphokines, and immunoglobulins.

38. A process according to claim 28 wherein said protein is an immunoglobulin.

REMARKS

Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information.

Applicant respectfully requests the entry of the above preliminary amendment.

Examiner is invited and encouraged to contact the undersigned if such contact would facilitate prosecution of this application.

Respectfully submitted,

Date: June 20, 2001

Virginia C. Bennett
Reg. No. 37,092

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CERTIFICATE OF EXPRESS MAILING (37 CFR 1.10)
I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Assistant Commissioner of Patents Washington, D.C. 20231 on June 20, 2001

Marilyn Eldridge

Production of Proteins

The present invention concerns the production of proteins by cell culture techniques and in particular concerns new and improved processes for the production of proteins useful in therapeutic and diagnostic applications.

The commercial production of proteins by cell culture particularly for use in medical applications remains a costly exercise principally due to the relatively low levels of proteins (particularly so-called "rare proteins") produced by many cell types. One approach to addressing this problem is the use of agents to induce cells to produce higher than normal amounts of the desired protein. An example of such an agent is butyric acid or, more typically, a salt thereof such as sodium butyrate, which is believed to modify gene expression at a molecular level through its effect on the methylation state of DNA, which in turn affects transcriptional gene activation. The inducing agent supplements the culture media during the culturing process and is typically following a period of cell culturing. During the process the culture media including the inducing agent may be changed by either a continuous process in which new media is continually added as old medium is removed or in a batch type process in which some of the medium is removed from the cells and replaced.

In EP 0 239 292 B1, NB1/19 cells were incubated with a variety of sodium butyrate concentrations ranging from 0.1mM to 1.0mM for a period of only approximately 8 days. In WO 89/06686, a process of enhancing protein production by cultured eukaryotic cells through the addition of butyric acid or salt thereof in concentrations of 0.1mM to 10.0mM is suggested. In this disclosure, cells are first grown to confluence before the addition of the acid or salt. This is generally in line with accepted thinking that butyric acid, whilst effective in promoting protein production, also has a potentially deleterious

effect on the viability of cells, making it prudent to bring cells to confluence or to a relatively high cell population, before exposing them to the butyric acid.

In US 5,705,364, the use of an alkanoic acid or salt thereof at concentrations of between 0.1mM and 20mM in optional conjunction with osmotic control to specifically increase silac acid content of glycoproteins is discussed. In Nucleic Acids Res., 1983, 11, no.21 the effects of sodium butyrate on DNA-mediated gene transfer is investigated at concentrations ranging from 2 to 10mM. In Can.Res, Vol.46, Feb 1986, 713-716, an investigation into the effects of sodium butyrate on the synthesis and methylation of DNA in normal cells and their transformed counterparts is disclosed. In this investigation, concentrations ranging from 5 to 100mM are used. In US 5,378,612, a culture medium for culturing transformed cells is disclosed. The culture media may contain butyric acid at an exemplified concentration of 1mM.

The present inventors have found, contrary to conventional expectations, that the use and maintenance of unusually low concentrations of an alkanoic acid in culture media comprising culturing cells, leads to enhanced protein production without, over an extended period of time, significantly affecting cell viability. Furthermore, the low alkanoic acid concentration permits the presence of alkanoic acid in the culturing media at an earlier stage in the culturing process than hitherto described.

In accordance with the present invention, we provide a process for the production of a protein by cell culture which comprises the step of culturing eukaryotic cells which constitutively produce e.g. secrete, said protein in a culture media which media comprises an alkanoic acid and/or salt thereof at a maintained concentration of less than 0.1mM.

In accordance with the present invention, we provide a process for the production of a protein by cell culture which comprises the steps of (a)

culturing eukaryotic cells which constitutively produce e.g. secrete said protein in a culture media which media comprises an alkanoic acid and/or salt thereof at a concentration of less than 0.1mM (b) subculturing the cell culture (c) supplementing the subculture media with additional alkanoic acid and/or salt thereof to maintain the concentration therein during the culturing process at less than 0.1mM.

Novel proteins obtained by the process of the present invention as hereinbefore described also forms an aspect of the present invention.

It will be understood by those skilled in the art that the terms "maintained concentration" and "to maintain the concentration" does not necessarily imply that the concentration in the culturing media must be kept constant since a certain degree of variation in concentration is permissible whilst still achieving the same or similar results. Of course, Zero mM is excluded from the term "less than 0.1mM".

In accordance with the present invention we provide a process for the production of a protein by cell culture which comprises the steps of (a) culturing eukaryotic cells which constitutively produce e.g. secrete said protein in a culture media which comprises an alkanoic acid and/or salt thereof at a specified concentration which concentration is less than 0.1mM.

Eukaryotic cells useful in the practice of the present invention may be e.g. yeast or animal and be anchorage dependent or independent. Preferably, the cells are mammalian e.g. rat, mouse and hamster. Cells of the present invention may be immortalised cells. Immortalised cells may be transformed or transfected with exogenous DNA e.g. via a plasmid, coding for the desired protein according to techniques standard and well known to those skilled in the art. Cells for use in the present invention include hybridoma cells e.g.

hybrid cells produced by fusion of antibody producing cells with myeloma cells. The process of the present invention maybe used in the culturing of hybridoma cells for the production of immunoglobulins e.g. antibodies.

Cells useful in the present invention include NS0 cells (non-immunoglobulin secreting mouse myeloma B cells), and CHO cells (Chinese hamster ovaries). Preferably, cells of the present invention are derived from stable cell lines, the production of which is well known to those skilled in the art. For example, stable cell line expression may be produced using a glutaminine synthetase gene amplification system (such as commercially available from Celltech). Briefly, linearized expression vectors containing cDNA encoding e.g. hamster glutamine synthetase, under the control of an Early promoter such as SV40 and splicing and polyadenylation signals and cDNA of the desired protein, e.g. antibody heavy and light chains are introduced into mammalian cells by electroporation. Transfected cells are then selected for the ability to grow in, for example, a glutamine free medium. See Bebbington *et al*, 1992, Biotechnology 10, 169-175 to which the reader is specifically referred.

Proteins which maybe produced by the present invention include therapeutically and/or diagnostically useful eukaryotic proteins which may be naturally occuring or artificial (e.g. fusion proteins). Examples of proteins whose production may benefit from the present invention include hormones, for example growth hormone e.g. human growth hormone, enzymes, enzyme inhibitors or lymphokines. The process of the present invention is particularly useful in the production of immunoglobulins, e.g. naturally occurring and artificial (chimeric and humanised) antibodies or analogues or fragments thereof

Suitable fragments thereof include Fab, Fv fragments and single chain antibodies. Antibodies maybe polyclonal or more preferably monoclonal from any suitable class or subclass. It is preferred that the antibody is a IgG,

particularly IgG_1 antibody or fragment derived therefrom. Preferably such proteins are suitable for use in the therapeutic (including prophylactic) or diagnostic treatment of human diseases, e.g. pro-inflammatory disorders such as rheumatoid arthritis, osteoarthritis, or other disorders. Thus in the case of antibodies, the antibody may be produced in a humanised form.

The alkanoic acid or salt thereof of the present invention, is preferably a straight chain C_{2-10}, especially C_{3-6} and in particular is butyric acid or metal salt thereof, e.g. sodium butyrate. Systems and processes for the detection of alkanoic acids and salts, e.g. chromatography, will be readily apparent to those skilled in the art. Preferably, the acid and/or salt thereof is kept at a concentration in the cell culture media, which whilst less than 0.1mM, is greater than 0.025mM, preferably greater than 0.05mM.

In a preferred embodiment, particularly for NS0 cells, the acid and/or salt thereof is present within the cell culture media at a concentration of about 0.075mM. These concentrations provide useful protein production with, over an extended period, minimal adverse effect on cell viability. Furthermore, the production of protein by cells exposed to these concentrations appears less erratic over time than at higher concentrations, for example 0.1mM. This is beneficial in providing greater consistency during any harvesting of protein that may take place during the culturing process. Furthermore a degree of control is provided over the process which, it will be appreciated, is desirable during commerical manufacturing processes.

It will be apparent to those skilled in the art that routine experimentation by e.g. simple titre experiments, may be employed to empirically determine the concentration or range of concentrations of acid and/or salt thereof in which, for a given cell type, enhanced protein production is evident with minimal adverse affect on cell viability particularly over an extended time period as measured by e.g. a simple viability count.

In accordance with the present invention we provide a process for the production of a protein in cell culture which comprises the steps of culturing eukarytoic cells which constitutively produce e.g. secrete said protein in a culture media which media comprises an alkanoic acid and/or salt thereof at a concentration, particularly less than 0.1mM, which concentration being sufficient to promote e.g. increase, protein production in the cells with minimal, preferably no, adverse affect on cell viability particularly when the cells are cultured for an extended period, e.g. greater than 10 days, more preferably greater than 30 days, even more preferably, greater than 50 days.

It is preferred that the concentration of the acid and/or salt within the culture media is maintained at approximately the same concentration during the culturing process (the specified concentration), preferably during a major proportion of the culturing process, more preferably, the entire time in which the acid and/or salt is present within the culture media. Where variation in concentration does occur, suitably such variation is within ± 20mM, aptly, ± 10mM, of the specified concentration.

The cell culture media of the present invention maybe comprise serum (e.g. animal serum such as fetal calf serum) or be serum free and may further comprise the usual components found in standard cell culture media. Those skilled in the art will appreciate that in circumstances where e.g. a glutamine dependency assay is employed, the culture media should comprise little, preferably, no, glutamine. The acid and/or salt is preferably admixed with the culture media prior to, at the start or shortly after commencement of the culturing process.

It is particularly preferred that the acid and/or salt thereof supplements the media following a short period from commencement in which the cells are cultured in the absence of acid and/or salt thereof to facilitate establishment

of a protein-producing (i.e stable) cell population. It is preferred that the cells are cultured for the minimum period of time necessary to establish the protein producing population prior to the addition of acid and/or salt. The short period may be determined empirically for a given cell line, for example, for NS0-GS cell line, the short period is about 4 days.

Maintenance of the concentration of the acid and/or salt thereof may be achieved by supplementing the media with additional acid and/or salt thereof by a fed-batch type process (in which cells and the culture medium are supplied to a culture vessel initially and additional culture nutrients are fed continously, or in discrete increments, to the culture during the culturing process) or alternatively through a continuous (i.e. perfusion) process in which new media is continually added as old medium is removed.

In preferred forms, a portion of the culture is subcultured by e.g.a draw-fill process wherein a portion of the culture is removed during the culturing process, the culture media adjusted and culturing of the remaining cells continued. The subculture is supplemented with additional acid and/or salt to adjust and therefore maintain the concentration therein at less than 0.1mM. The entire culturing process preferably takes place over an extended time course, i.e. greater than 10 days, preferably, greater than 40 days.

Preferably, the acid and/or salt is continuously present within the culture media during at least a portion of the culturing process. In particular, it is preferred that the acid and/or salt thereof is continuously present in the culture media over a major proportion of the culturing process, most preferably, the entire process.

Examples of culturing systems that may be employed in the present invention include fluidized bed bioreactors, hollow fiber bioreactors, roller bottle culture or stirred tank bioreactors. Generally, the culturing of cells

comprises submerging the cells (at a density of e.g. 0.2 x 10^5/ml) in the culture media contained within a suitable vessel. The media comprising cells and the acid and/or salt is then exposed to appropriate temperature (e.g. 36°c), pressure, pH, humidity and other conditions, over a period of time.

The process of the present invention may be used in conjunction with other techniques for increasing protein production in culturing cells, e.g. osmotic stressing of the culturing cells.

The desired protein may be isolated or recovered from the cell culture by conventional separation techniques. This may take place during or following termination of the culturing process. Such conventional techniques include centrifugation to remove particulate cell debris wherein the supernatant collected after the centrifugation step is treated by e.g. ultrafilitration, fractionation on immunoaffinity or ion-exchange columns or other chromatographic techniques and subjected to further purification techniques to purify, if desired, to homogeneity.

Proteins produced by the present invention may be used in the manufacture of pharmaceutical compositions which comprise an effective amount of the protein together with other constituents such as a pharmaceuticallly acceptable carrier as known and called for by accepted pharmaceutical practice. Such other constituents may include other therapeutic agents. Determination of the effective amount may be ascertained by routine experimentation and observation as known to those skilled in the art.

Suitably, such pharmaceutical compositions are made available in unit dosage form. Pharmaceutical compositions maybe in any form appropriate for the route of administration which, it will be appreciated, is dictated in part by the prevailing circumstances, e.g. condition to be treated. Thus, pharmaceutical compositions may be in the form of capsules, cachets,

tablets, powders or granules, suspensions in a sterile aqueous or non-aqueous liquid, onitment, paste or paint. Pharmaceutcal compositions may be used in conjunction, e.g. as part of a treatment regimen, with other therapeutic and/or diagnostic agents. It is particularly envisaged that proteins of the present invention are used in the therapeutic and/or diagnostic treatment of mammals including humans.

The present invention will now be illustrated, by way of example only, and with reference to the following figures, in which:

Fig.1 illustrates the comparison of antibody production in NS0 cell culture when cultured in media supplemented with five concentrations of sodium butyrate (0~0.1mM) over a period of 56 days. The following reference numerals denote concentration used:
(1): 0mM sodium butyrate
(2): 0.025mM sodium butyrate
(3):0.05mM sodium butyrate
(4):0.075mM sodium butyrate
(5):0.1mM sodium butyrate.

Fig.2 illustrates enhancement of antibody production by NS0 cells cultured in 0.075mM sodium butyrate in repeated batch culture. The following reference numerals denote:

(10): viability of cells without butyrate supplementation (control)
(11) viability of cells supplemented with sodium butyrate (0.075mM)
(12) antibody titre of control cells (10)
(13) antibody titre of cells (11)

Example 1

Five Erlenmeyer flasks were inoculated with recombinant murine NS0 cells transfected with a IgG_1 humanised anti-CD23 antibody (produced according to the method of Bebbington et al, as *supra*) in a cholesterol containing protein free/glutamine free medium at $0.2x10^5$cells/ml. Sodium butyrate (0.5M) in PBS was added to the flasks at the following concentrations: 0mM; 0.025mM; 0.05mM; 0.075mM and 0.10mM and were incubated at 36°C and 100rpm in a shake incubator for 4 days. Subsequently, the flasks were subcultured every four days to a start count of $0.2x10^5$ cells/ml, maintaining sodium butyrate concentrations as above in each flask. The flasks were sampled and assayed before each subculture. The culture was continued in this 'draw-fill'/'repeated batch' manner for 56 days, maintaining sodium butyrate concentrations at the concentrations indicated throughout. Viable cells were counted for each sample taken as outlined below. Antibody titre for each sample taken was determined as outlined below.

Viable cell count

Culture was diluted as necessary in culture medium. It was examined microscopically in a haemocytometer (Neubauer counting chamber) using Erythrosin B (Sigma) 0.04% w/v in PBS, pH 7 as an exclusion dye. The number of viable and non-viable cells per milliliter and the percentage viability of the culture was calculated.

Antibody titre

The quantity of Human IgG was determined by a Nephelometric method (Tanford, C. (1961) Light Scattering. Physical Chemistry of Macromolecules. New York:Wiley, 275-316; Whicher J.T et al (1978), " An evaulation of Hyland laser nephelometer PDQ system for the measurement of immunoglobulins"; Ann.Clin.Biochem. 15, p77-85), whereby the formation of insoluble immune-precipitin of Human IgG with highly specific antibody to Human IgG was

measured. The Nephelometer measured the intensity of the light scattered by the insoluble immune-precipitin in reaction solution. The change in intensity of the scattered light signal is proportional to the concentration of Human IgG in tested sample. The quantity of Human IgG in the sample is measured from a standard curve constructed from known concentration of purified Human IgG versus the rate of light scatter signal.

Results

Fig.1 illustrates a plot of antibody titre over time at various butyrate concentrations. Butyrate concentrations of 0.1mM and 0.075mM demonstrated a marked increase in antibody titre over control following 56 day culture. Butyrate concentrations of 0.1mM demonstrated erratic protein production during the culturing process whereas at 0.075mM a more consistent production was observed.

Fig.2 illustrates a plot of antibody production by viable cell count over 56 day culture. Cells cultured in the presence of butyrate at 0.075mM showed a slight decrease in viable cell count during the early stages of the culturing process which decrease had, by 56 day, narrowed considerably when compared to control. This may indicate that NS0 cells cultured in butyrate (0.075mM) develop a degree of adaptation or tolerance to the presence of butyrate at around this concentration. Antibody titre at 0.075mM showed a marked increase over control.

Claims

1. A process for the production of a protein by cell culture, said process comprising culturing eukaryotic cells which constitutively produce said protein in a medium which comprises an alkanoic acid and/or salt thereof at a maintained concentration of less than 0.1mM.

2. A process for the production of a protein by cell culture which comprises the steps of:
   (a) culturing enkaryotic cells which costitutively produce said protein in a culture medium which medium comprises an alkanoic acid and/or salt thereof at a concentration of less than 0.1mM.
   (b) subculturing the cell culture and
   (c) supplementing the subculture media with additional alkanoic acid / or salt thereof to maintain the concentration therein during the culturing process at less than 0.1mM.

3. A process according to claim 1 or 2, wherein the alkanoic acid and/or salt thereof is a straight chain C_{2-10}.

4. A process according to claim 3, wherein the straight chain is C_{3-6}.

5. A process according to claim 3 or 4, wherein the alkanoic acid is butyric acid or a metal salt thereof.

6. A process according to claim 3 or 4, wherein the salt is sodium butyrate.

7. A process according to any of the above claims, wherein the acid and/or salt thereof is less than 0.1mM but greater than 0.025mM.

8. A process according to claim 7, wherein the acid and/or salt thereof is less than 0.1mM but greater than 0.05mM.

9. A process according to claims 7 and 8, wherein the acid and/or salt thereof is about 0.075mM.

10. A process according to any of the above claims, wherein the eukaryotic cells may be NSO or CHO.

11. A process according to any of the above claims wherein the protein produced by said process may be selected from hormones, enzymes, enzyme inhibitors, lymphokines or immunoglobulins.

12. A process according to claim 11, wherein the protein is an immunoglobulin.

FIG. 1

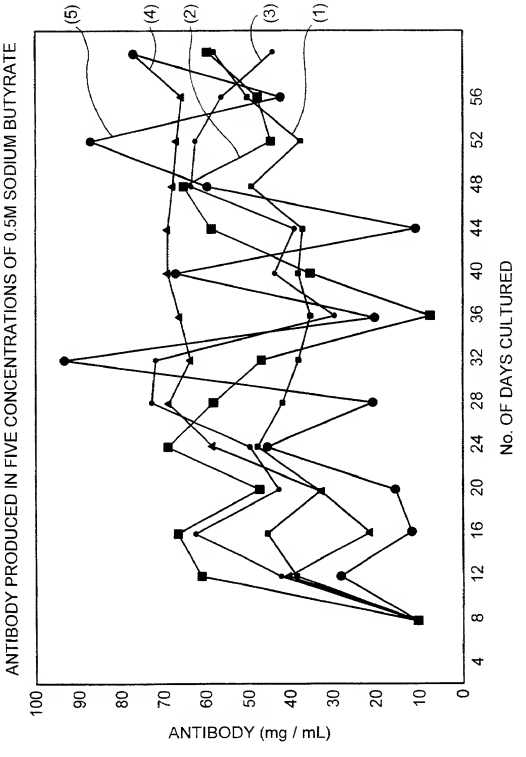
ANTIBODY PRODUCED IN FIVE CONCENTRATIONS OF 0.5M SODIUM BUTYRATE
(5)
(4)
(2)
(3)
(1)
ANTIBODY (mg / mL)
0
10
20
30
40
50
60
70
80
90
100
No. OF DAYS CULTURED
4
8
12
16
20
24
28
32
36
40
44
48
52
56

FIG. 2

EFFECT OF SODIUM BUTYRATE ON NS0-GS IN CONTINUOUS CULTURE FOR 56 DAYS

09/868,666

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

STOTT, Michael, J.
GlaxoSmithKline
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 16 août 2001 (16.08.01)	
Applicant's or agent's file reference PG3635	**IMPORTANT NOTIFICATION**
International application No. PCT/EP99/10157	**International filing date** (day/month/year) 21 décembre 1999 (21.12.99)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address	State of Nationality	State of Residence
STOTT, Michael, J. Glaxo Wellcome plc Glaxo Wellcome House Berkeley Avenue Greenford Middlesex UB6 0NN United Kingdom		
	Telephone No. 0171-493-4060	
	Facsimile No. 0181-966-8838	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address	State of Nationality	State of Residence
STOTT, Michael, J. GlaxoSmithKline Corporate Intellectual Property Two New Horizons Court Brentford Middlesex TW8 9EP United Kingdom		
	Telephone No. 020 8966 8412	
	Facsimile No. 020 8966 8838	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☐ the International Preliminary Examining Authority
☐ the designated Offices concerned
☒ the elected Offices concerned
☐ other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Raissi
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY

ATTORNEY'S DOCKET
PG3635USW
First Names Inventor
Islam
Complete if known:
App No.:
09/868,666
Filing Date
June 20, 2001
Group Art Unit:

( ) Declaration submitted with initial filing or

(x)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PRODUCTION OF PROTEINS

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[ ] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number PCT/EP99/10157 filed December 21 1999 and was amended on (MM/DD/YYYY)
_____(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. 9828624.8	GB	12/23/1998	x
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
1.		
2.		
3.		

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PU3635USW

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

David J. Levy	Reg. No. 27,655	James P. Riek	Reg. No. 39,009	Bonnie L. Deppenbrock	Reg. No. 28,209
Charles E. Dadswell	Reg No 35,851	Virginia C. Bennett	Reg. No. 37,092	John L. Lemanowicz	Reg. No. 37,380
Karen L. Prus	Reg No. 39,337	Frank P.Grassler	Reg. No. 31,164	Amy H. Fix	Reg. No. 42,616
Robert H. Brink	Reg. No. 36,094	Christopher P. Rogers	Reg. No. 36,334		
Elizabeth Selby	Reg No. 38,298	Lorie Ann Morgan	Reg. No. 38,181		

Send Correspondence to:
David J. Levy, Patent Counsel
Corporate Intellectual Property Department
GlaxoSmithKline, Inc.
Five Moore Drive, PO Box 13398
Research Triangle Park, NC 27709

23347
PATENT TRADEMARK OFFICE

Direct Telephone Calls to:
Virginia C. Bennett
919-483-1012

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2	FULL NAME OF INVENTOR	FAMILY NAME: **ISLAM**	FIRST GIVEN NAME: **Seema**	SECOND GIVEN NAME/INITIAL:
0	**INVENTOR'S SIGNATURE**	Seema Islam.		**DATE:** 19 Nov. 2001.
1	RESIDENCE & CITIZENSHIP	CITY: **New York**	STATE OR FOREIGN COUNTRY: **New York**	COUNTRY OF CITIZENSHIP: **GB**
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **715 Park Avenue**	CITY: **New York** NY	STATE & ZIP CODE/COUNTRY: **NY 10021 US**
2	FULL NAME OF INVENTOR	FAMILY NAME: **SHARP**	FIRST GIVEN NAME: **Nigel**	SECOND GIVEN NAME/INITIAL: **Alan**
0	**INVENTOR'S SIGNATURE**			**DATE:**
2	RESIDENCE & CITIZENSHIP	CITY: **Stevenage**	STATE OR FOREIGN COUNTRY: **Hertfordshire GB**	COUNTRY OF CITIZENSHIP: **GB**
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline, Inc. Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME/INITIAL:
0	**INVENTOR'S SIGNATURE**			**DATE:**
3	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
	POST OFFICE ADDRESS	POST OFFICE ADDRESS:	CITY:	STATE & ZIP CODE/COUNTRY:

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY

( ) Declaration submitted with initial filing or

(x)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))

ATTORNEY'S DOCKET PG3635USW
First Names Inventor: **Islam**
Complete if known: App No.: 09/868,666
Filing Date June 20, 2001
Group Art Unit:

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PRODUCTION OF PROTEINS

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[ ] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number PCT/EP99/10157 filed December 21 1999 and was amended on (MM/DD/YYYY) _____(if applicable)

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Application No.	Filing Date (MM/DD/YYYY)	
1.		
2.		
3.		
4.		
5.		

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PU3635USW

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

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Charles E. Dadswell	Reg. No. 35,851	Virginia C. Bennett	Reg. No. 37,092	John L. Lemanowicz	Reg. No. 37,380
Karen L. Prus	Reg. No. 39,337	Frank P.Grassler	Reg. No. 31,164	Amy H. Fix	Reg. No. 42,616
Robert H. Brink	Reg. No. 36,094	Christopher P. Rogers	Reg. No. 36,334		
Elizabeth Selby	Reg. No. 38,298	Lorie Ann Morgan	Reg. No. 38,181		

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2	FULL NAME OF INVENTOR	FAMILY NAME: **ISLAM**	FIRST GIVEN NAME: **Seema**	SECOND GIVEN NAME/INITIAL:
0	INVENTOR'S SIGNATURE			DATE:
	RESIDENCE & CITIZENSHIP	CITY: **Barnet**	STATE OR FOREIGN COUNTRY: **Hertfordshire GB**	COUNTRY OF CITIZENSHIP: **GB**
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME: **SHARP**	FIRST GIVEN NAME: **Nigel**	SECOND GIVEN NAME/INITIAL: **Alan**
0	INVENTOR'S SIGNATURE			DATE: August 8th, 2001
	RESIDENCE & CITIZENSHIP	CITY: **Stevenage**	STATE OR FOREIGN COUNTRY: **Hertfordshire GB** (GB)	COUNTRY OF CITIZENSHIP: **GB**
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline, Inc. Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME/INITIAL:
0	INVENTOR'S SIGNATURE			DATE:
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS:	CITY:	STATE & ZIP CODE/COUNTRY: